Studies of the Effects of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin on Mammalian Hepatic δ-Aminolevulinic Acid Synthetase

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Introduction

The toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), a contaminant formed during the manufacture of the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) is well known (*1-4*). The widespread utilization of this compound has caused increased concern about the potential health hazards created by the presence of TCDD in the environment. The articles which accompany this account, indeed, attest to the highly toxic nature of TCDD in both laboratory animals and man.

Interest in TCDD as a potential porphyrogenic agent arose when porphyria cutanea tarda, a form of hepatic porphyria, occurred in industrial workers associated with the manufacture of 2,4,5-T (*5*). Hepatic porphyria is a syndrome characterized by a variety of symptoms including the overproduction and excretion of porphyrins, pigmentation of the skin, photosensitivity, and intestinal and neurological disorders. The disease is characterized biochemically by an increase in the activity of the mitochondrial enzyme δ-aminolevulinic acid (ALA) synthetase, which is the first and rate-limiting enzyme in the heme biosynthetic pathway (*6*) (Fig. 1). The possibility that TCDD has porphyrogenic properties has been indicated by the recent observation that TCDD is a potent inducer of hepatic ALA synthetase in chick embryos (*7*). There is, however, no evidence to indicate that TCDD produces similar effects in mammalian species. The chick embryo system is exquisitely sensitive to the effects of agents which induce ALA synthetase (*8*). Previous studies from our laboratory (*9, 10*), on the other hand, have shown that mammalian species demonstrate a striking variability in their response to porphyric agents, especially at different stages of development.

These studies were undertaken, therefore, to determine the possible porphyrogenicity of TCDD in mammalian species and to assess further the utility of the chick embryo liver system as an indicator of the potential porphyrogenic effects of environmental agents in mammals.

Materials and Methods

ALA synthetase activity was determined by two procedures. In liver homogenates

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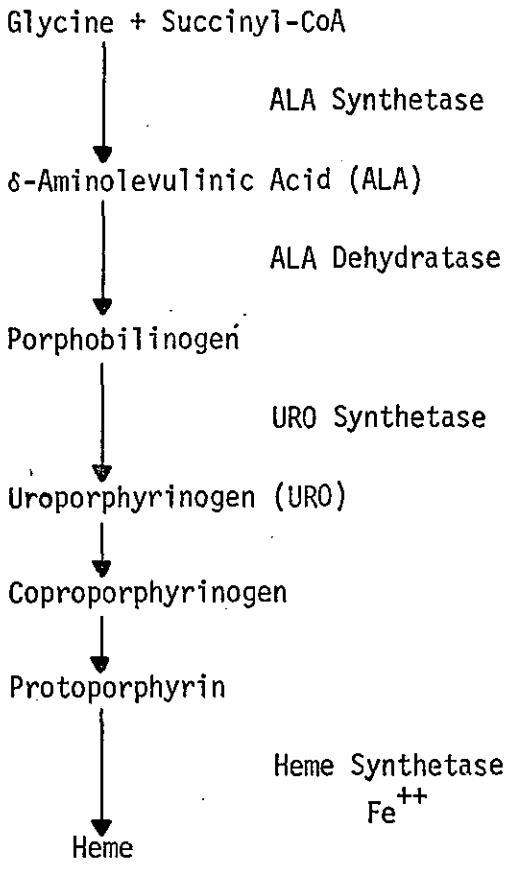

FIGURE 1. Heme biosynthetic pathway.

ALA synthetase activity was assayed using the ion exchange chromatography technique described by Mauzerall and Granick (*11*), with liver homogenates prepared for incubation as described by Marver et al. (*12*). ALA synthetase activity in subcellular fractions and at various stages of enzyme purification was measured using the procedure described by Scholnick et al. (*13*). In the latter case the incubation medium was modified to include $10^{-4}M$ GTP in addition to the prescribed substrates.

The method of Scholnick was also used for the isolation and 50-fold purification of ALA synthetase from porphyric rat liver. Hepatic subcellular fractions were prepared as described by Hayaski et al. (*14*). Protein concentrations were assayed by the method of Lowry et al. (*15*).

All test animals were treated orally with a standard solution of TCDD (10 μg/ml) dissolved in a corn oil/acetone mixture (6:1) 24 hr prior to sacrifice unless otherwise indicated. Two groups of control animals were used in all *in vivo* experiments. The first group received only the corn oil/acetone mixture in an amount equivalent to that in which TCDD was administered to test animals. The second group was treated subcutaneously with allylisopropylacetamide (AIA) (400 mg/kg), which is a well known and potent inducer of ALA synthetase in mammals (*12, 16*).

TCDD was obtained from Dow Chemical Company, Midland, Michigan. AIA was a gift from Hoffmann-LaRoche, Nutley, New Jersey. All animals and other chemicals were obtained from standard sources.

Results and Discussion

Initial studies were designed to determine the potential porphyrogenic effects in rats of TCDD when administered in doses up to 25 μg/kg, the reported LD_{50} for this species (*17*). Male rats were treated with a single 5 or 25 μg/kg dose of TCDD, and hepatic ALA synthetase activity was assayed at periods up to 28 days thereafter. In animals receiving a single dose of AIA, ALA synthetase activity increased to approximately seven times the control level after 24 hr and returned to control levels by the third day after treatment. On the other hand, TCDD did not significantly alter ALA synthetase activity, as measured in whole liver homogenates, during any part of the test period in any of the animals. All measurements of enzyme activity were within the range observed in controls.

It has been recently determined (*14*) that ALA synthetase is a mitochondrial enzyme but is synthesized extramitochondrially on the cytoplasmic ribosomes. The enzyme is subsequently incorporated into the mitochondria, where it becomes active. ALA synthetase activity may be altered by agents which interfer with any aspect of this process. It was, therefore, of interest to determine if TCDD might influence the subcellular localization of ALA synthetase and thereby alter the regulation of hepatic hem-

Table 1. Subcellular distribution of hepatic ALA synthetase in normal and AIA- and TCDD-treated rats.[a]

Group	Treatment	ALA, nmole/mg protein-hr ± S.E.			
		Mitochondrial fraction	9,000*g* supernatant	105,000*g* supernatant	Microsomal fraction
1	Corn Oil	0.52 ± 0.09	0.27 ± 0.08	0.24 ± 0.10	0.03 ± 0.01
2	AIA	2.26 ± 0.80	1.44 ± 0.40	1.47 ± 0.30	0.28 ± 0.10
3	TCDD	0.58 ± 0.10	0.34 ± 0.06	0.15 ± 0.43	0.01 ± 0.01
4	AIA + TCDD	2.91 ± 0.87	1.37 ± 0.51	1.17 ± 0.51	0.27 ± 0.08

[a] Rats were treated with TCDD (25 μg/kg) and/or AIA (400 mg/kg) 24 hr prior to sacrifice.

atopoiesis in a manner which could not be detected when ALA synthetase activity was measured in whole liver homogenates. The results of these experiments are seen in Table 1. Analysis of the subcellular distribution of ALA synthetase in adult rat liver reveals a 2:1 distribution of activity between mitochondrial and postmitochondrial fractions. Most of the postmitochondrial activity is retained in the 105,000*g* supernatant fraction with very little found in the microsomes. Control rats in the group treated with AIA showed substantial increases in the ALA synthetase activity in all fractions but relatively little alteration in the distribution of activity between fractions after 24 hours. TCDD administered alone at 25 μg/kg doses or together with AIA caused no significant alteration of ALA synthetase activity from that observed in control groups. In addition, TCDD did not affect the induction of ALA synthetase by AIA nor alter the subcellular distribution of the enzyme during induction. Similar observations were made when TCDD was administered in 100 mg/kg doses or when these experiments were conducted with female rats or mice.

In vitro tests of the possible effects of TCDD or ALA synthetase activity were conducted in liver homogenates, isolated mitochondria and on ALA synthetase purified 50-fold from porphyric rat liver. In enzyme incubation mixtures containing TCDD in concentrations ranging from 10^{-9} to $10^{-6}M$ no discernable effects on the enzyme activity could be observed.

Finally, the potential porphyrogenic effects of TCDD during the perinatal period were investigated. These studies were conducted in fetal rats which were 3 days from delivery, (−3 day), and on two groups of newborn rats, 4 and 12 days after delivery on the day of sacrifice. Newborn rats were treated orally with 25 μg/kg doses of TCDD, whereas fetal rats were treated by way of the mother. Livers from mother rats served as adult samples. The results are shown in Table 2. In no case did ALA synthetase activity in TCDD-treated rats differ from that observed in untreated animals. Fetal ALA synthetase is typically five to eight times that of the adult (*9*) and declines to adult levels shortly after birth. Refractoriness to induction of ALA synthetase is observed until the activity approaches that of the adult (*10, 18*). At no stage of development, however, did the ALA synthetase activity in TCDD-treated rats significantly differ from that observed in controls.

These results are of particular interest in view of the potent induction of ALA synthetase activity produced by TCDD in the chick embryos. The lack of a significant effect of TCDD on ALA synthetase in mammals suggests that major differences exist among these species in the biological mechanisms which determine the ultimate pharmacological disposition of chemicals such of TCDD. Species variations in drug distribution, biotransformation, and excretion are well known (*19*). On the other hand, major differences in the mode of action of TCDD may also reflect alterations in the regulation

Table 2. Study of potential effects of TCDD on ALA synthetase during development in rats.[a]

Group	Treatment	ALA, nmole/hr-g liver ± S.E.			
		−3 day	+4 day	+12 day	Adult
1	Corn oil	241 ± 31	94 ± 7	50 ± 16	45 ± 5
2	TCDD	253 ± 47	76 ± 21	60 ± 8	44 ± 13
3	AIA	248 ± 28	118 ± 12	113 ± 13	347 ± 17

[a] Pregnant and newborn rats were treated 24 hr prior to sacrifice with TCDD (25 μg/kg) or AIA (400 mg/kg).

of hepatic heme synthesis in these species. Striking variations in the developmental aspects of ALA synthetase regulation have already been described. The chick embryo liver is one of the most sensitive systems available in which to study the induction of ALA synthetase (*8*). In contrast, our previous studies (*10*) have shown that fetal mammals are totally insensitive to the effects of drugs which induce or repress hepatic ALA synthetase in chick embryo or in adult animals. Moreover, fetal mammalian ALA synthetase activity is significantly elevated in comparison with that of the adult (*9*), whereas the control level of ALA synthetase in 17-day old chick embryo liver is only one-third that measured in the adult chicken (*20*).

Differences in porphyrin metabolism in these two species is also suggested by the failure of a wide variety of drugs which induce hepatic porphyrin accumulation in chick embryos to do so in mammalian liver (*21*). Among the drugs capable of causing significant increases in porphyrin levels in chick embryo liver are 3,5-dicarbethoxy-1,4-dihydrocollidine (DDC), glutethimide, methsuximide, secobarbital, methylprylon, and mephenytoin. Of this group, only DDC is capable of producing porphyrin accumulation in mice. In addition, it has been recently indicated that uroporphyrinogen synthetase, another enzyme in the heme biosynthetic pathway, may play a rate-limiting role in hepatic heme synthesis in certain strains of mice (*22*).

It therefore appears that significant differences exist among these species with regard to various steps in the regulation of hepatic heme synthesis and porphyrin metabolism. These differences, along with variations in the capacity to metabolize and distribute drugs such as TCDD, may account for the differences in susceptibility of these species to the porphyrogenic effects of TCDD and perhaps to other environmental contaminants.

The foregoing considerations attest to the increasingly prevalent observation that many environmental agents are hazardous not only by virtue of their inherent toxicity but also because of their specificity of action in different species. While utilization of non-mammalian test systems may provide, in some cases, a sensitive indication of the potentially toxic effects of certain drugs and chemicals in mammals, this study demonstrates the necessity for developing test procedures which will more clearly predict the deleterious effects of environmental contaminants in mammalian species, especially in man.

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